

Remarks:**Rejection under 35 U.S.C. § 112:**

Claim 5 is rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner alleges that no support is seen for the amendment which replaces “the hydrogen atom of the hydroxyl group” with -- the hydrogen atom at all or independently of each other at any of the hydroxyl groups--. Applicants have amended claim 5 to cancel the matter to which the Examiner alludes to and Applicants respectfully request that the 35 U.S.C. § 112, first paragraph, rejection with respect to claim 5 be reconsidered and withdrawn.

Claims 5-10 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner alleges that claim 5 is unclear because the body of claim 5 does not support the preamble of claim 5 regarding the treatment of arteriosclerosis. Applicants have amended claim 5 to include the phrase “a subject in need of treatment for arteriosclerosis” in the body of claim 5 which should obviate the 35 U.S.C. § 112 rejection, second paragraph rejection. Accordingly, Applicants respectfully request that the 35 U.S.C. § 112, second paragraph rejection with respect to claim 5 and dependent claims 6-10 be reconsidered and withdrawn.

Rejection under 35 U.S.C. § 103(a):

Claims 5-10 are again rejected, as set forth in the Office Action mailed December 12, 2002, under 35 U.S.C. § 103(a) as being unpatentable over Murase *et al.* in view of Cynshi *et al.* Further to Applicant’s remarks in response to the 35 U.S.C. 103(a) rejection of the Office Action mailed December 12, 2003, Applicants again emphasize that many factors are believed to be associated with the crisis and formation of arteriosclerosis, and the free radicals and the active oxygen radicals are only part of these factors. Though a

compound has anti-oxidant activity, it is not necessarily effective in the treatment of arteriosclerosis. This is self-evident because no anti-oxidant agent has yet been authorized as a pharmaceutical preparation for treating arteriosclerosis.

Enclosed herewith is a declaration under 37 C.F.R. § 1.132. In the experiments, the effects of the chromal glucoside (TMG) on the expression of the cell adhesion molecules (VCAM-1 and ICAM-1) were investigated. The cell adhesion molecule is one of the important factors in the development of arteriosclerosis. For example, refer to “III. FACTORS FOR THE ARTERIOSCLEROSIS”, PAGE 87-97 of “THE EXPERIMENTAL MEDICINE SERIES FOR THE CLINICIAN NO. 15, THE MOLECULE MEDICINE OF THE ARTERIOSCLEROSIS”, enclosed.

As is clear from the results of the experiments, TMG suppresses the increase expression of VCAM-1 and ICAM-1 on Human aortic endothelial cells (HAEC) induced by the cytokine IL-1 β . Consequently, this not only proves that TMG has anti-oxidant properties but also that TMG expresses inhibitory effects on cell adhesion molecules greatly associated with the action mechanism of arteriosclerosis.

Murase *et al.* in view of Cynshi *et al.* neither teaches nor suggests that chromal glucoside possesses the inhibitory effects of cell adhesion as well as anti-oxidant properties as discussed above.

Based on the foregoing, Applicants respectfully request that the 35 U.S.C. §103(a) rejection be reconsidered and withdrawn with respect to claims 5-10.

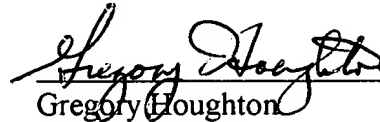
Conclusion:

In view of the foregoing, Applicants submit that all pending claims are in condition for allowance and request that all claims be allowed. The Examiner is invited to contact the undersigned should he believe that this would expedite prosecution of this application. It is believed that no fee is required. The

Commissioner is authorized to charge any deficiency or credit any overpayment to
Deposit Account No. 13-2165

Respectfully submitted,

Dated: January 5, 2004

A handwritten signature in cursive script, appearing to read "Gregory Houghton", written over a horizontal line.

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動脈硬化に関与する諸因子

白血球-血管内皮細胞間の接着分子

—単球の粥状動脈硬化病巣への集簇機構における役割—

久米典昭

粥状動脈硬化の初期像は単球・マクロファージ由来の泡沫細胞の血管内皮下での局在的な集簇である。血中単球の血管壁内への侵入は、血管内皮細胞表面に発現される白血球に対する特異的な接着分子の関与が考えられる。現在では複数の接着分子による多段階の機序が想定されるが、少なくともVCAM-1およびICAM-1が実際に粥状動脈硬化の初期病変に局在して発現され、またこれらの分子の発現誘導機構も明らかになりつつある。

1. 粥状動脈硬化における単球の役割

粥状動脈硬化発生の初期には、細胞内に大量のエステル化コレステロールを蓄積した泡沫細胞 (foam cells) と呼ばれる細胞の血管内皮下での局在的な集簇がみられる。この泡沫細胞の起源は、血中単球 (monocytes) 由来のマクロファージ (macrophages) および血管平滑筋細胞であるといわれているが、特にその初期の病変では大部分がマクロファージ由来であるとされる。マクロファージは酸化などの変性 (modification) を受けた低比重リポ蛋白 (low density lipoprotein: LDL) を、その特異的な受容体を介して取り込むなどの機構により泡沫細胞となり、脂肪線条 (fatty streak) と呼ばれる病変を形成する。さらにマクロファージは、サイトカイ

268 : 2126-2133, 1993

- 20) Kita, T., Nagano, Y., Yokode, M., Ishii, K., Kume, N., Ooshima, A., Yoshida, H. & Kawai, C. : Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA*, 84 : 5928-5931, 1987
- 21) Kita, T., Brown, M. S., Bitheimer, D. W. & Goldstein, J. L. : Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoprotein in WHHL rabbits. *Proc. Natl. Acad. Sci. USA*, 79 : 5963-5967, 1982
- 22) Van Lenten, B. J., Fogelman, A. M., Jackson, R. L., Shapiro, S., Haberland, M. E. & Edwards, P. A. : Receptor-mediated uptake of remnant lipoproteins by cholesterol-loaded human monocyte-macrophages. *J. Biol. Chem.*, 260 : 8783-8788, 1985
- 23) Takahashi, S., Kawarabayashi, Y., Nakai, T., Sakai, J. & Yanamoto, T. : Rabbit very low density lipoprotein receptor : A low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl. Acad. Sci. USA*, 89 : 9252-9256, 1992
- 24) Yokode, M., Pabak, R. K., Hammer, R. E., Brown, M. S., Goldstein, J. L. & Anderson, R. G. W. : Cytoplasmic sequence required for basolateral targeting of LDL receptor in livers of transgenic mice. *J. Cell Biol.*, 117 : 39-46, 1992

放出することなどにより、血管平滑筋細胞の内膜への遊走、増殖を伴ったより複雑な病変へと進行させる。また粥状動脈硬化巣にはTリンパ球が存在することが知られており、Tリンパ球由来のサイトカインなどが病態に関与している可能性も考えられる。このような単球、リンパ球の血管壁内への侵入にはどのようなメカニズムが関与するのであろうか³¹⁻³³⁾。

2. 粥状動脈硬化巣への単球の侵入機構における接着分子仮説

高コレステロール食を負荷されたラット、サルなどの実験動物のモデルにおいて、コレステロール負荷を開始して早期の、まだ明らかな粥状動脈硬化病変のみられない時期に、すでに多数の単球が動脈内皮の一部に局所的に付着している像が走査電顕にて観察されている³⁴⁾。これは単球の内皮下への侵入に先立つ血管内皮細胞への接着という現象が重要な役割を演じているものと推測することができ、そして、このような血管内皮細胞への接着という現象の少なくとも一部は、内皮細胞表面の単球に対する接着性の局所的な変化によるものであり、細胞表面に発現される白血球に対する特異的な接着分子を介するものである可能性を考慮することができる。

3. 血管内皮細胞に発現される白血球接着分子

血管内皮細胞における白血球に対する接着分子については、おもに炎症組織への種々の白血球の集積、あるいはリンパ球のリンパ節へのホーミング(homing)を支えるものとしてその分子機構の解明が進められてきた。現在では複製の異なる接着分子が同定され、それらの分子構造、そして白血球側のリガンドも明らかにされている(図1)。これらは、その分子構造の類似性から、セレクチン

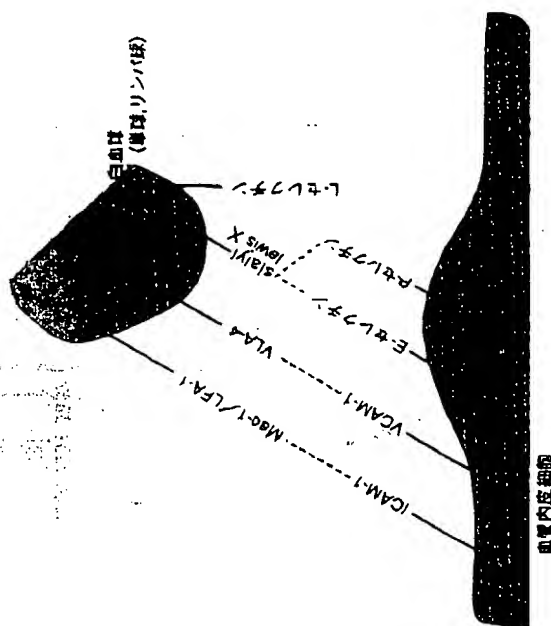


図1 血管内皮細胞-白血球間の接着を支える接着分子

(selectin)と免疫グロブリンスーパーファミリー(immunoglobulin superfamily)とに大別される³⁵⁻³⁷⁾。

1 セレクチン

セレクチンはレクチン様ドメイン、EGF (epidermal growth factor, 上皮増殖因子)様ドメイン、コンセンサスリピートを細胞外にもつ構造をとる膜蛋白であり、E-セレクチン(endothelial leukocyte adhesion molecule-1: ELAM-1)、L-セレクチン(leukocyte adhesion molecule-1: LAM-1, lectin adhesion molecule-1: LECAM-1)、P-セレクチン(granule membrane protein-140: GMP-140, CD62)の3種の類似の構造をもつ分子が見出されている。E-セレクチンはIL-1(interleukin-1)、TNF(tumor necrosis factor, 腫瘍壊死因子)などのサイトカインおよびエンドキシンにより刺激された血管内皮細胞にのみその発現が認められ、その発現調節はおもに転写のレベルにあり、転写因子の1つであるNF- κ Bの活性化が必要であるが、そのみでは十分ではないといわれている³⁸⁾。P-セレクチンは血管内皮細胞および血

ことも明らかにされている²³⁰⁾

ICAM-1 (intercellular adhesion molecule-1)²³¹⁾は、血管内皮細胞に限らず種々の細胞でその発現が認められる。培養血管内皮細胞では、すでに少量の発現が認められるが、IL-1, TNF, インターフェロン γ (IFN- γ) などのサイトカインあるいはエンドトキシン²³²⁾の刺激により、さらにその発現が誘導される。ICAM-1は、 β 2 サブユニットをもつインテグリンである LFA-1 (lymphocyte function-related antigen 1) (CD11a/CD18) および Mac-1 (CD11b/CD18) をそのリガンドとする。

VCAM-1 および ICAM-1 の白血球側のリガンドである VLA-4 および LFA-1, Mac-1 は恒常的に発現されているが、これらインテグリンの VCAM-1, ICAM-1 への結合性 (avidity) は、白血球に対する走化因子あるいは C キナーゼ (protein kinase C) を活性化するホルボールエステルなどにより増強されるといわれる²³³⁾。SHUIM

4. 白血球の血管内皮細胞への接着機構 —その多段階モデル—

流速のある条件下での白血球のサイトカインで刺激された培養血管内皮細胞への接着には、インテグリンと ICAM-1 との関与は少なく、L-セレクチンとそのリガンドとの接着により支えられることが示されている。また、このことはサイトカインなどの刺激にて誘導される L-セレクチンに対するカウンター受容体の存在を示唆する²³⁴⁾。in vivo においても、サイトカインで刺激された、露出された腸間膜静脈を通過する蛍光標識された白血球の挙動を顕微鏡下で観察することにより、L-セレクチンが白血球の血管内皮細胞表面での転がりがながらの緩やかな接着状態 (ローリング) を支えるものであること、さらにローリングに引き続く強固な接着はインテグリンと ICAM-1 とにより支えられるといわれる²³⁵⁾ (図 2)。動脈中の内皮細胞においての直接的な証拠は現在のところ得られては

小板にてその発現が認められる。P-セレクチンは血管内皮細胞では Weibel-Palade body、血小板では α 顆粒に蓄えられ、トロンビン、ヒスタミンあるいはカルシウムイオノフォアなどの刺激により速やかに細胞膜表面に運ばれる。L-セレクチン²³⁶⁾は、単球、リンパ球を含む種々の白血球表面に恒常的に発現されている。L-セレクチンは白血球に対する走化因子 (chemoattractant) あるいはホルボールエステルなどの刺激により、白血球表面から速やかに分断 (shedding) されるといわれる²³⁷⁾。セレクチンのリガンドは sialyl lewis X と呼ばれる糖鎖²³⁸⁾であり、セレクチンのレクチン様ドメインに結合すると考えられる。しかしながら、白血球と血管内皮細胞との間の接着には結合部位であるレクチン様ドメインだけでなく、正常な細胞質内ドメインが必要であり、おそらくはセレクチンと細胞骨格との間の連絡が重要であるものと推察される²³⁹⁾。

2 免疫グロブリンスーパーファミリー

VCAM-1 (vascular cell adhesion molecule-1, intercellular adhesion molecule-110 : INCAM-110)²⁴⁰⁾は、IL-1, TNF, IL-4 などのサイトカインおよびエンドトキシンにより発現が誘導される²⁴¹⁾が、少なくとも TNF による発現誘導は転写のレベルであり、NF- κ B の活性化を必要とする²⁴²⁾が、そのみでは不十分といわれる²⁴³⁾。VCAM-1 のリガンドは VLA-4 (very late antigen-4) と呼ばれる α 4 β 1 インテグリンである。VLA-4 はまたフィブロネクチンに対する受容体でもあるが、VCAM-1 の結合部位はフィブロネクチンの結合部位とは同一ではないといわれる²⁴⁴⁾。VLA-4 は白血球細胞ではリンパ球、単球などで発現されるが、好中球では発現されないため、VCAM-1 はよりリンパ球および単球に選択的な接着分子といえる。ヒト VCAM-1 は、当初 6 個の免疫グロブリン様ドメインをもつ構造が報告されたが、ヒトで実際に発現されている大部分は 7 個のドメインをもつ分子といわれ、これらの違いは alternative splicing に由来するものといわれる²⁴⁵⁾。また、7 個のドメインをもつ分子には 2 カ所の VLA-4 結合部位が存在するが、6 個のドメインをもつ分子ではドメイン 4 を欠くためしか所である

6. 粥状動脈硬化において VCAM-1、ICAM-1 の発現を誘導する刺激

VCAM-1 および ICAM-1 はサイトカイン、エンドトキシンによりその発現が誘導される。従って、特に進行した病変においては、血管壁内に侵入した単球、T リンパ球などが産生、放出する IL-1、IL-4 などのサイトカインにより、これらの接着分子の発現がさらに増幅されている可能性も考えられる。しかしながら、これらの単球、リンパ球の侵入を最初に支える接着分子の発現を誘導する刺激は何なのだろうか。ウサギの粥状動脈硬化モデルにおいてきわめて早期より認められる変化は、LDL などのリポ蛋白濃度の血管分岐部など、粥状動脈硬化の好発部位の動脈壁内での局所的な増加といわれる²¹⁾。また LDL の酸化変性 (oxidative modification) が粥状動脈硬化の病因として重要な役割を演じていることは、酸化 LDL に対するモノクローナル抗体を用いた免疫組織化学により、粥状動脈硬化巣において酸化 LDL が検出されること²²⁾、*in vitro* で LDL の酸化変性を抑制する抗酸化剤の投与が *in vivo* で WHHL ウサギの粥状動脈硬化の進展を阻止すること²³⁾ などより、現在では広く受け入れられている。従って、血管内皮下に蓄積した酸化変性を受けた LDL が、直接に血管内皮細胞を刺激し接着分子などの発現を誘導していると考えられることは困難でない。

LDL の酸化変性に伴いその粒子中に含まれるリン脂質であるホスファチジルコリン (phosphatidylcholine) が、加水分解を受けリゾホスファチジルコリン (lyso-phosphatidylcholine: lyso-PC) となること、そしてこの lyso-PC が単球に対する走化因子であるといわれていた²⁴⁾。われわれはこのリン脂質が培養動脈内皮細胞を刺激し、ICAM-1 および VCAM-1 の発現を mRNA のレベルで誘導することを示した²⁵⁾。このようにサイトカイン以外の刺激による接着分子の誘導機構が、粥状動脈硬化病巣への単球集積を促す最初の刺激の1つである可能性が示唆される。また、リゾホスファチジルコリンは酸化 LDL および β 超低比重リポ蛋白 (VLDL) といっ

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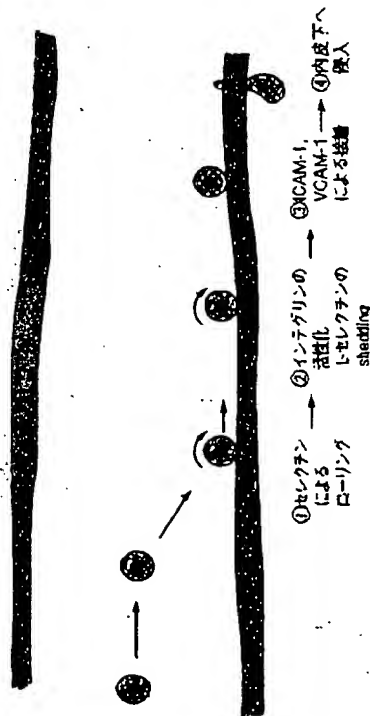


図2 血管内皮細胞への白血球接着機構における多段階モデル

いないが、同様の多段階の機構が関与する可能性も考えられる。

5. 粥状動脈硬化巣で発現される白血球-血管内皮細胞間の接着分子

コレステロール負荷ウサギおよび家族性高コレステロール血症の動物モデルである WHHL ウサギでは、VCAM-1 が早期の泡沫細胞病変を被う内皮細胞に限局して発現されていることが見出されている²⁶⁾。ウサギ VCAM-1 はコレステロール負荷を開始して1週間前後のまだ明らかな泡沫細胞病変のみられない時期の大動脈において、すでにその発現が認められることより、粥状動脈硬化発生のきわめて早期における単球集積への関与が示唆される²⁷⁾。一方、ヒトの粥状動脈硬化巣においては、泡沫細胞により多くの ICAM-1 の発現が認められるといわれる²⁸⁾。また、ウサギ大動脈のバルーン障害による内膜肥厚モデルにおいても、VCAM-1 および ICAM-1 の発現が認められ²⁹⁾、さらにウサギ頸動脈の電気刺激による別の内膜肥厚モデルにおいて、抗 CD18 抗体の投与が単球の内皮下への侵入を完全ではないが部分的に抑制するとの報告もある³⁰⁾。

たatherogenic なリポ蛋白にて著明に増加が認められるだけでなく、炎症組織においても細胞外に放出されるホスホリパーゼ (phospholipase) A₂ の作用によりその増加が認められ¹⁰⁾、粥状動脈硬化ばかりではなく炎症組織への白血球の動員にも関与している可能性も考えられる。

「おわりに」

現在では、複数の白血球-血管内皮細胞間の接着分子を介する多段階の分子機構が明らかにされている。粥状動脈硬化病変の動脈内皮への単球、リンパ球の接着機構も、おそらくは炎症組織への白血球募集機構と同様な接着分子を介する多段階のメカニズムを推定することができるが、さらに多くの解明されるべき器が残されている。

文 献

- 1) Gimbrone, M. A. Jr., Kume, N. & Cybulsky, M. I. : Vascular endothelial dysfunction and the pathogenesis of atherosclerosis. In *Atherosclerosis reviews*. (Weber, P.C. & Leaf, A. eds.) Raven Press, New York, NY, 1993
- 2) Ross, R. : The pathogenesis of atherosclerosis : a perspective for the 1990s. *Nature*, 362 : 801-809, 1993
- 3) Steinberg, D., Parthasarathy, S., Carew, T.E. et al. : Beyond Cholesterol : Modification of low density lipoprotein that increases its atherogenicity. *N. Engl. Med.*, 320 : 915-924, 1989
- 4) Libby, P. & Hansson, G.K. : Involvement of immune system in human atherosclerosis : current knowledge and unanswered questions. *Lab. Invest.*, 64 : 5-15, 1991
- 5) Springer, T.A. : Adhesion receptors of the immune system. *Nature*, 346 : 425-434, 1990
- 6) Osborn, L. : Leukocyte adhesion to endothelium in inflammation. *Cell*, 63 : 3-6, 1990
- 7) Springer, T.A. & Lasky, L.A. : Sticky sugars for selectins. *Nature*, 349 : 196-197, 1991
- 8) Bevilacqua, M.P., Stengelin, S., Gimbrone, M.A. Jr. et al. :

Endothelial leukocyte adhesion molecule-1 : An inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science*, 243 : 1160-1165, 1989

- 9) Montgomery, K.F., Osborn, L., Hession, C. et al. : Activation of endothelial-leukocyte adhesion molecule 1 (ELAM-1) gene transcription. *Proc. Natl. Acad. Sci. USA*, 88 : 6523-6527, 1991
- 10) Johnston, G.L., Cook, R.G. & McEyer, R.P. : Cloning of GMP-140, a granule membrane protein of platelets and endothelium : Sequence similarity to proteins involved in cell adhesion and inflammation. *Cell*, 56 : 1033-1044, 1989
- 11) Tedder, T.F., Isaacs, C.M., Ernst, T.J. et al. : Isolation and chromosomal localization of cDNAs encoding a novel human lymphocyte cell surface molecule, LAM-1. *J. Exp. Med.*, 170 : 123-133, 1989
- 12) Lasky, L.A., Singer, M.S., Yednock, T.A. et al. : Cloning of a lymphocyte homing receptor reveals a lectin domain. *Cell*, 56 : 1045-1055, 1989
- 13) Kishimoto, T.K., Jutila, M.A., Berg, E.L. et al. : Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. *Science*, 245 : 1238-1241, 1989
- 14) Kansas, G.S., Ley, K., Munro, J.M. et al. : Regulation of leukocyte rolling and adhesion to high endothelial venule through the cytoplasmic domain of L-selectin. *J. Exp. Med.*, 177 : 833-838, 1993
- 15) Osborn, L., Hession, C., Tizard, R. et al. : Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds lymphocytes. *Cell*, 59 : 1203-1211, 1989
- 16) Rice, G.E. & Bevilacqua, M.P. : An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science*, 246 : 1303-1306, 1989
- 17) Rabinowitz, S.S., Cybulsky, M.I., Kume, N. et al. : Endothelial-dependent mechanisms of monocyte adhesion. In *Fifth Leiden Conference on Mononuclear phagocytes*. (Furth, R.V. ed.) Kluwer Academic Publishers, Leiden, The Netherlands, p81, 1991
- 18) Neish, A.S., Williams, A.J., Palmer, H.J. et al. : Functional analysis of the human vascular cell adhesion molecule 1 promoter. *J. Exp. Med.*, 176 : 1583-1594, 1992
- 19) Elices, M.J., Osborn, L., Takada, Y. et al. : VCAM-1 on activated endothelium interacts with leukocyte integrin VLA-4/fibronectin binding site. *Cell*, 60 : 577-584, 1990
- 20) Cybulsky, M.I., Fries, J.W.U., Williams, A.J. et al. : Alternative splicing of human VCAM-1 in activated vascular endothelium. *Am.*

- J. Pathol., 138 : 815-820, 1991
- 21) Cybulsky, M.I., Fries, J.W.U., Williams, A. et al. : Gene structure, chromosomal location, and basis for alternative mRNA splicing of the human VCAM-1 gene. *Proc. Natl. Acad. Sci. USA*, 88 : 7859-7863, 1992
 - 22) Vonderhede, R.H. & Springer, T.A. : Lymphocyte adhesion through very late antigen 4 : Evidence for novel binding site in the alternatively spliced domain of vascular cell adhesion molecule 1 and an additional $\alpha 4$ integrin counterreceptor on stimulated endothelium. *J. Exp. Med.*, 175 : 1433-1442, 1992
 - 23) Osborn, L., Vassallo, C. & Benjamin, C.D. : Activated endothelium binds through a novel binding site in the alternatively spliced domain of vascular cell adhesion molecule-1. *J. Exp. Med.*, 176 : 99-107, 1992
 - 24) Staulon, D.E., Marlin, S.D., Stratowa, C. et al. : Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell*, 52 : 925-933, 1988
 - 25) Simons, D., Makgoba, M.W. & Seed, B. : ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. *Nature*, 331 : 624-627, 1988
 - 26) Shimizu, Y., van Seventer, G.A., Horgan, K.J. et al. : Regulated expression and binding of three VLA ($\beta 1$) integrin receptors on T cells. *Nature*, 345 : 250-253, 1990
 - 27) Spertini, O., Luscoskas, F.W., Gimbrone, M.A. Jr. et al. : Monocyte attachment to activated human vascular endothelium in vitro is mediated by leukocyte adhesion molecule-1 (L-selectin) under nonstatic conditions. *J. Exp. Med.*, 175 : 1789-1792, 1992
 - 28) Ley, K., Gachigens, P., Fennie, C. et al. : Lectin-like cell adhesion molecule 1 mediates leukocyte rolling in mesenteric venules in vivo. *Blood*, 77 : 2553-2555, 1991
 - 29) von Andrian, U.H., Chambers, J.D., McEvoy, L.M. et al. : Two-step model of leukocyte-endothelial cell interaction in inflammation : Distinct roles for VCAM-1 and the leukocyte β_2 integrins *in vivo*. *Proc. Natl. Acad. Sci. USA*, 88 : 7538-7542, 1991
 - 30) Butcher, E.C. : Leukocyte-endothelial cell recognition : three (or more) steps to specificity and diversity. *Cell*, 67 : 1033-1036, 1991
 - 31) Cybulsky, M.I. & Gimbrone, M.A. Jr. : Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science*, 251 : 788-791, 1991
 - 32) Li, H., Cybulsky, M.I., Gimbrone, M.A. Jr. et al. : An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Arterioscler. Thromb.*, 13 : 197-204, 1993
 - 33) Poston, R.N., Haskard, D.O., Coucher, J.R. et al. : Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *Am. J. Pathol.*, 140 : 665-673, 1992
 - 34) Tanaka, H., Swanson, S. J., Lee, C. et al. : Sustained activation of vascular cells and leukocytes in rabbit aorta after balloon injury. *Circulation*, 86 : suppl. 1-86, 1992
 - 35) King, D., Fingerle, L. & Hartan, J.M. : Inhibition of Leukocyte extravasation with a monoclonal antibody to CD18 during formation of experimental intimal thickening in rabbit carotid arteries. *Arterioscler. Thromb.*, 12 : 997-1007, 1992
 - 36) Schwenke, D.C. & Carew, T.E. : Initiation of atherosclerotic lesions in cholesterol-fed rabbits. I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. *Arteriosclerosis*, 9 : 895-907, 1989
 - 37) Kita, T., Nagano, Y., Yokode, M. et al. : Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA*, 84 : 5928-5931, 1987
 - 38) Quinn, M.T., Partasarathy, S. & Steinberg, D. : Lysophosphatidylcholine : a chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc. Natl. Acad. Sci. USA*, 85 : 2805-2809, 1988
 - 39) Kume, N., Cybulsky, M.I. & Gimbrone, M.A. Jr. : Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J. Clin. Invest.*, 90 : 1138-1144, 1992
 - 40) Vadas, P. & Pruzanski, W. : Role of secretory phospholipase A_2 in the pathobiology of disease. *Lab. Invest.*, 55 : 391-404, 1986

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(Translation of Page 87-97)

III. FACTORS FOR THE ARTERIOSCLEROSIS

ADHESION MOLECULE BETWEEN LEUKOCYTE AND VASCULAR ENDOTHELIAL 5 CELL

- Role of Monocyte in Mechanism of Confluence at Focus of
Atherosclerosis -

Noriaki Kume

10 The initial image of atherosclerosis (pultaceous
arteriosclerosis) manifests itself in the local confluence
of foam cells which originate in monocytes/macrophages under
the vascular endothelium. The penetration of monocytes in
blood into the vascular wall is thought to be participated
in by an adhesion molecule peculiar to the leukocyte expressed
15 on the surfaces of vascular endothelial cells. At present,
in spite of the assumption of the multistage mechanism
involving a plurality of adhesion molecules, at least VCAM-1
and ICAM-1 are actually expressed as localized in the initial
morbid change of atherosclerosis and the mechanism of inducing
20 the expression of these molecules has been being elucidated.

1. Role of Monocyte in Atherosclerosis

During the initial stage of the evolution of
atherosclerosis, the local confluence under the vascular
endothelium of foam cells which have a large amount of
25 esterified cholesterol accumulated therein is observed. In
spite of the assertion that these foam cells originate in
the macrophages arising from monocytes in blood and the
vascular smooth cells, it is held that particularly the initial
morbid change originates mostly in macrophages. The
30 macrophages transform into the foam cells through the
mechanism of introjecting low density lipoprotein (LDL)
modified as by oxidation through the medium of their peculiar

receptors and consequently form a morbidity called fatty streak. Further, the macrophages, by yielding and emitting cytokines or growth factors, are aggravated to a more complicated morbid change accompanied by migration and propagation of vascular smooth cells in the intine. Further, the focus of atherosclerosis is known to allow the presence of T-lymphocytes therein. It is conceivable that the cytokines which originate in T-lymphocytes possibly participate in this morbid change. What mechanism participates in the penetration of monocytes and lymphocytes into the vascular wall?^{1) - 4)}

2. Hypothesis of Adhesion Molecule in Mechanism of Penetration of Monocyte into Atherosclerosis

In the models of such experimental animals as rats and monkeys which had been loaded with hypercholesterol feed, an image of local adhesion of numerous monocytes to part of the arterial endothelium was observed under a scanning electron microscope already in the early stage following the start of the loading of cholesterol, i.e. when no clear sign of the morbid change of atherosclerosis was visible.²⁾ From this fact, it can be inferred that the phenomenon of adhesion of monocytes to the vascular endothelial cells prior to the penetration thereof to below the endothelium plays an important role. It is conceivable that at least part of the phenomenon of such adhesion to the vascular endothelial cells is caused by the local change of adhesiveness to the monocytes on the surfaces of endothelial cells possibly through the medium of an adhesion molecule peculiar to the leukocytes expressed on the surfaces of cells.

3. Leukocyte Adhesion Molecule Expressed on Vascular Endothelial Cells

As regards the adhesion molecule for the leukocytes in

the vascular endothelial cells, efforts have been chiefly directed toward elucidating the molecular mechanism responsible for supporting the confluence of various species of leukocytes at inflammatory tissues or the homing of lymphocytes to the lymphonodus. So far, a plurality of different adhesion molecules have been identified and their molecular structures and their ligands on the leukocytes' side have been elucidated (Fig. 1). These adhesion molecules are broadly divided by analogy of molecular structure into selectin and immunoglobulin superfamily.^{5) - 7)}

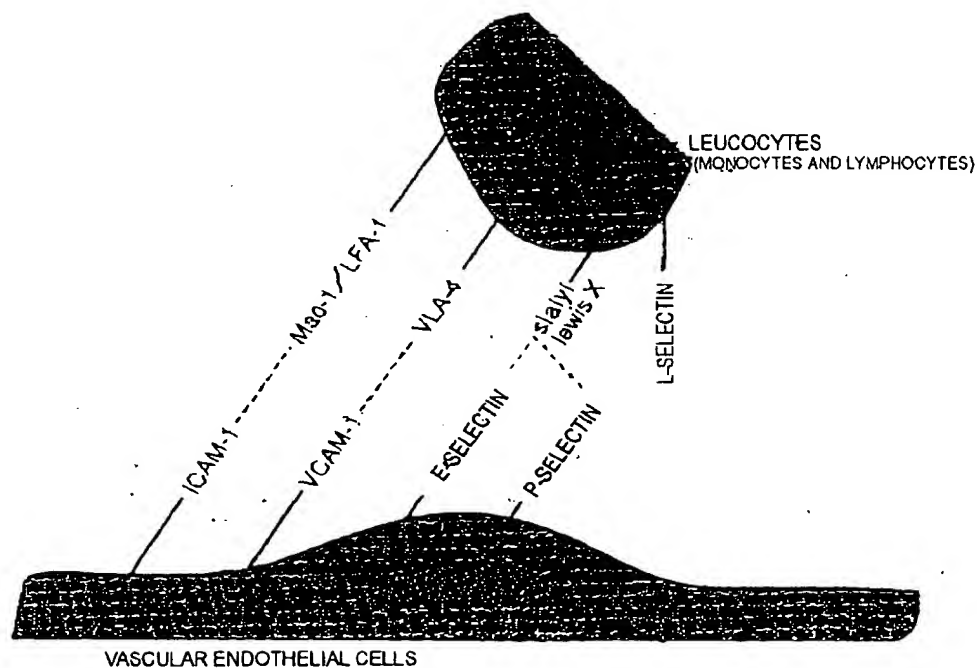


FIG. 1 ADHESION MOLECULES SUPPORTING ADHESION BETWEEN VASCULAR ENDOTHELIAL CELLS AND LEUCOCYTES

1. Selectin

The selectin is a protein of such a structure as

extracellularly retains a lectin-like domain, an EGF (epidermal growth factor)-like domain, and a consensus repeat. It has been found in three similar structures, i.e. E-selectin (endothelial leukocyte adhesion molecule-1: ELAM-1),
5 L-selectin (leukocyte adhesion molecule-1: LAM-1, lectin adhesion molecule-1: LECAM-1), and P-selectin (granule membrane protein-140: GMP-140, CD62). The E-selectin⁸⁾ is recognized to be expressed only in the vascular endothelial cells stimulated by such cytokines as IL-1 (interleukin-1)
10 and TNF (tumor necrosis factor) and endotoxin. The control of this expression is mainly on the level of transcription and is in need of activation of NF- κ B which is one of the transcription factors. It is, however, said that the activation alone does not suffice the control. The
15 P-selectin¹⁰⁾ is recognized to be expressed to vascular endothelial cells and blood platelets. The P-selectin is stored in the Weibel-Palade body in the vascular endothelial cells and in the α granules in the blood platelets and is speedily transmitted to the surfaces of cell membranes by
20 the stimulation of thrombin, histamine, or calcium ionophore. The L-selectin¹¹⁾¹²⁾ is constantly expressed on the surfaces of various species of leukocytes including monocytes and lymphocytes. The L-selectin is possessed of such a control mechanism which enables it to be speedily shed from the surfaces
25 of leukocytes by the stimulation of a chemoattractant or a phorbol exerted on leukocytes.¹³⁾ The ligand of the selectin is a saccharic chain⁷⁾ called "sialyl lewis X" and is considered to be joined to the lectin-like domain of selectin. The adhesion between leukocytes and vascular endothelial cells
30 necessitates not only the lectin-like domain which is a binding site but also the normal cytoplasmic inclusion domain. It is presumed that the linkage between the lectin and the

cytoskeleton is probably important.¹⁴⁾

2. Immunoglobulin superfamily

The VCAM-1 (vascular cell adhesion molecule-1, intercellular adhesion molecule-110: INCAM-110)¹⁵⁾¹⁶⁾ has the expression thereof induced by such cytokines as IL-1, TNF, and IL-4 and endotoxin.¹⁷⁾ The induction of the expression by at least the TNF, however, is on the level of transcription and is in need of activation of NF- κ B. It is said that the activation alone does not suffice the induction. The ligand of the VCAM-1 is an $\alpha 4/\beta 1$ integrin called "VLA-4 (very late antigen-4)." Though the VLA-4 is a receptor for fibronectin, it is said that the binding site of the VCAM-1 is not identical with the binding site of the fibronectin.¹⁹⁾ Since the VLA-4 is expressed in lymphocytes and monocytes and not in neutrophils so far as blood cells are concerned, it is safe to infer that the VCAM-1 is an adhesion molecule more selective for lymphocytes and monocytes. The human VCAM-1 was at first reported to possess a structure having six immunoglobulin-like domains. Most human VCAM-1 expressed actually in man, however, is said to be a molecule having seven domains. This difference is said to originate in alternative splicing.²⁰⁾²¹⁾ Further, it has been demonstrated that the molecule having seven domains permits the presence of VLA-4 binding sites at two positions and the molecule having six domains permits the presence at one position on account of the lack of domain 4.²²⁾²³⁾

The ICAM-1 (intercellular adhesion molecule-1)²⁴⁾²⁵⁾ is recognized to be expressed not only in the vascular endothelial cells but also in various species of cells. In the cultured vascular endothelial cells, the expression is already recognized in a small amount and this expression is further induced by the stimulation of such cytokines as IL-1, TNF,

and interferon (IFN- γ) or endotoxin. The ICAM-1 has LFA-1 (lymphocyte function-related antigen 1) (CD11a/CD18) and Mac-1 (CD11b/CD18), i.e. integrins possessing a $\beta 2$ subunit, as the ligands thereof.

5 The VLA-4 and the LFA-1 and the Mac-1 which are leukocyte sideligands of the VCAM-1 and ICAM-1 are constantly expressed. It has been known that the avidity of these integrins for the VCAM-1 and the ICAM-1 possess such a control mechanism that this avidity is enhanced by the chemoattractant for
10 leukocytes or the phorbol ester capable of activating protein kinase C.⁵⁾¹¹⁾²⁶⁾

4. Mechanism of Adhesion of Leukocyte to Vascular Endothelial Cells

- Multistage Model -

15 It has been shown that the adhesion of leukocytes to the stimulated cultured vascular endothelial cells under the condition involving flow velocity is not much concerned with the ICAM-1 but is supported by the adhesion of L-selectin to the ligand thereof. Further, this fact suggests the
20 presence of a counter receptor for the L-selectin which is induced by the stimulation such as of cytokines.²⁷⁾ Even in vivo, when the behavior of leukocytes marked by fluorescence which has been stimulated by cytokines and passed through an exposed minute mesenteric veins is observed under a
25 microscope, it is found that the L-selectin supports the state of moderate rolling adhesion on the surfaces of vascular endothelial cells and further that the strong adhesion which follows the rolling is supported by the integrin and the ICAM-1²⁸⁾⁻³⁰⁾ (Fig. 2). No direct proof in the artery side
30 endothelial cells has not been obtained to date. Nevertheless, the possibility of a similar multistage mechanism participating in this behavior is not inconceivable.

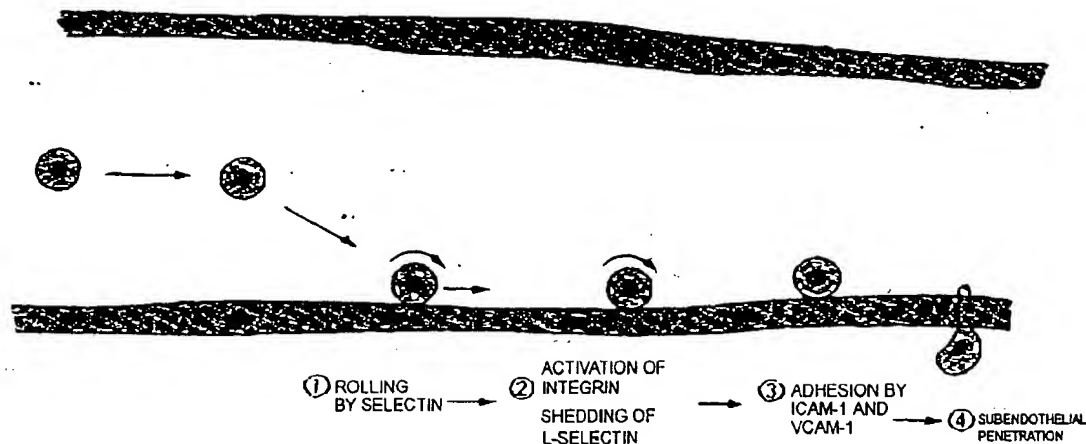


FIG. 2 MULTISTAGE MODEL IN MECHANISM OF ADHESION OF LEUCOCYTES TO VASCULAR ENDOTHELIAL CELLS

5. Adhesion Molecule between Leukocytes and Vascular Endothelial Cells Expressed in Locus of Atherosclerosis

It has been found that in cholesterol-loaded rabbits and WHHL rabbits which are animal models for familial hypercholesterolemia, the VCAM-1 is locally expressed in the endothelial cells covering the morbid foam cells.³¹⁾ The leporine VCAM-1 is recognized to be expressed already in the aorta roughly one week after the start of cholesterol loading, namely when no clear morbid change in foam cells is observed. This fact suggests that this adhesion molecule participates in the confluence of monocytes in the very early stage of the evolution of atherosclerosis.³²⁾ On the other hand, it is said that in the focus of human atherosclerosis, the foam cells are recognized to induce expression of ICAM-1 in a large amount.³³⁾ Also in the models of tylosis of inner membrane caused by forced passage of a balloon through the leporine

aorta, the VCAM-1 and the ICAM-1 are recognized to be expressed.³⁴⁾ Further, it is reported that in the other models of tylosis of inner membrane caused by the electric stimulation of the leporine carotid arteries, the administration of an anti-CD18 antibody results in partly, if not perfectly, repressing the subendothelial penetration of monocytes.³⁵⁾

6. Stimulation for Inducing Expression of VCAM-1 and ICAM-1 in Atherosclerosis

The VCAM-1 and the ICAM-1 have their expressions induced by cytokines and endotoxin. It is, therefore, conceivable that in particularly progressed morbid changes, the expression of these adhesion molecules is further amplified by such cytokines as IL-1 and IL-4 which are yielded and emitted by monocytes and T-lymphocytes permeating vascular walls.

What stimulation induces the expression of adhesion molecules which first support the penetration of such monocytes and lymphocytes? The changes which are recognized to occur very early in the models of leporine atherosclerosis are claimed to be local increases of the concentration of lipoprotein such as LDL in the arterial walls at an favorite site for atherosclerosis such as the site for vascular ramification.³⁾³⁶⁾ The assertion that the oxidative modification of LDL plays an important role as the cause for atherosclerosis has found widespread acceptance as evinced by the fact that the oxidative LDL is detected at the focus of atherosclerosis by the immunocyto-chemistry using a monoclonal antibody against oxidative LDL,³⁾ the fact that the administration of an antioxidant capable of repressing the oxidative modification of LDL in vitro results in inhibiting the development of atherosclerosis of WHHL rabbits in vivo,³⁾³⁷⁾ and so on. It is, therefore, not difficult to conclude that the LDL which has accumulated subendothelially

in the blood vessel and has undergone oxidative modification directly simulates the vascular endothelial cells and induces expression of adhesion molecules.

It has been heretofore held that the phosphatidylcholine, a phospholipid to be incorporated in the particles of LDL in consequence of the oxidative modification thereof, is transformed by hydrolysis into lyso-phosphatidylcholine (lyso-PC) and that this lyso-PC forms a chemoattractant for monocytes.³⁸⁾ We have demonstrated, however, that this phospholipid stimulates cultured vascular endothelial cells and induces expression of the ICAM-1 and the VCAM-1 on the mRNA level.³⁹⁾ The mechanism of inducing adhesion molecules by the stimulation caused by other than cytokines suggests that this stimulation may be one of the first stimulations that promote the confluence of monocytes at the focus of atherosclerosis. The lyso-phosphatidylcholine is recognized not only to be conspicuously increased by such atherogenic lipoproteins as an oxidized LDL and β very-low density lipoprotein³ but also to be increased by the action of phospholipase A₂ which is extracellularly expelled even in the inflammatory tissues.⁴⁰⁾ Thus, the possibility of this adhesion molecule participating in the mobilization of leukocytes in the inflammatory tissues is not inconceivable.

Conclusion

The multistage molecular mechanism which operates through the medium of a plurality of adhesion molecules between leukocytes and vascular endothelial cells is being elucidated today. The mechanism of the adhesion of monocytes and lymphocytes to the arterial endothelium suffering from a morbid change of atherosclerosis probably permits inference of a multistage mechanism which operates through the medium

of adhesion molecules similarly to the mechanism of the confluence of leukocytes at inflammatory tissues. This mechanism, however, entails riddles yet to be solved.

5 Bibliography(omitted)